General Variant Classification/ Assertion criteria

General Information

Variant identification and interpretation are critical steps in making genetic diagnosis and personalized medicine a reality. The in-house method of variant classification at K & H Personalized Medicine Clinic® complies with the American College of Medical Genetics (ACMG) standards. We devised standard internal guidelines to assess the robustness of publicly available information, gene-disease relationship, the clinical impact of nucleotide variations, the availability of treatments, and preventive measures. Internal criteria are designed to refine ACMG/AMP guidelines based on the latest data available for assessing the strength of the variant and the most recent information specific to genes & gene-phenotype association.

K & H Personalized Medicine Clinic® variant interpretation features a combination of open-source tools with automated in-house algorithms. A wide range of information from public resources and in-house databases is retrieved through machine learning approaches, and a team of qualified professionals does an in-depth evaluation.

In the assessment of the variant classification, K & H Personalized Medicine Clinic® considers information and evidence that includes, but is not limited to, the following 5 significant parameters. The functional impact of the gene in causing the disease phenotype, functional impact of variation in the gene product based on *in silico*, *in vitro,* and *in vivo* studies, variant-disease association, prevalence and significance. All information derived from peer-reviewed published literature, and in-house database testing, are considered when weighing a variant in favor of pathogenicity.

Variant Classification

K & H Personalized Medicine Clinic® classifies the variants into 6 categories as per ACMG guidelines and based on the above criteria. Variants are categorized under each of these categories based on the consistency of the evidence. It should be noted that documentation can make any early evidence obsolete.

1. ***Pathogenic variants***:

A variant that is more likely to cause disease is defined as a pathogenic variant. All the variants which can cause loss of protein function or predictable significant damage to the gene product or can alter protein/protein interactions fall under this category.

SNPs, Indels, and CNVs are the primary variant types considered. The modifier effects of the primary variants are then identified. Further, those affecting the gene product function, i.e., variants that could lead to truncation of the gene product such as nonsense variant, frameshift alterations, stop gain variants, and variants that can affect the canonical splice acceptor and splice donor sites, are classified as pathogenic. Such variants that have significant genotype-phenotype relationships and are highly prevalent in disease phenotype compared to unaffected individuals or control groups are also considered pathogenic. In-silico tools such as SIFT, Polyphen-2, CADD, and data from MutationTaster are utilized to predict the pathogenicity, strength, and impact of variation on protein function of the variant.

Likely pathogenic variants: Variants located in functional domains of the protein with high or moderate mutation impact and are frequently prevalent in observed phenotype compared to a healthy population, without demonstrated significance from case-control studies yet are categorized as likely-pathogenic. The frequency of prevalence is obtained from open source databases control data, peer-reviewed publications, and internal data reviews.

3. Risk factor variants:
Probably damaging missense variants, intronic variants, UTRs with moderate variant impact, and significant genotype-phenotype association is considered risk factor variant. These variants increase an individual's susceptibility or predisposition to a particular disease phenotype.

4. Benign:
Variants of low or moderate variant impact (from *in silico* studies) known to have a significant genotype-phenotype relationship as evident from in vitro and or in vivo studies and a not known to cause the disease are considered variants of benign clinical significance. In-frame deletions or insertions in repetitive regions without a known function are also regarded as variants of benign significance.

5. Likely Benign:
Any variants with conflicting interpretations of association with the disease phenotype are weighed carefully for the functional effect of the variant and the number of affected individuals when compared to a healthy population. If the variant is predicted to have low impact, have a low variant impact, is tolerated mutation in '*in silico'* observations with conflicting evidence, or has inadequate clinical information, they are classified as variants of likely benign clinical significance. Low allele frequency variants consistent with particular ethnic groups are also considered likely benign.

6. Uncertain significance:
Variants with insufficient evidence or multiple studies with opposing results for a given phenotype are classified as variants of unknown or uncertain significance. In-frame deletion alterations, Synonymous (intron variants other than splice site variants), and missense variations that have a low variant impact and do not have functional genotype-phenotype association are classified under variants of uncertain significance. Variants with known evidence of gene-phenotype relationship and no sufficient reported evidence on the variant-phenotype association are also categorized as Variants of Uncertain Significance. Variants with sufficient reported evidence of non-association with the phenotype from both internal and external sources, any novel silent variants which do not show the genotype-phenotype association, are also classified as Variants of Uncertain Significance.

For rare disorders, proportionally lower allele frequencies are accepted as stand-alone criteria relative to the disease incidence. Open source population databases like 1000 Genomes, NHLBI Exome Sequencing Project (ESP), Exome Variant Server, and Exome Aggregation Consortium (ExAC) are screened for frequency of the variant in a control population. Additional databases, computational tools, and in-house algorithms are tested and considered as new sources of information become available.

Extensive interactions between the variant classification team, molecular biologists, bioinformaticians, and genetic counselors ensure continual progress in variant classification quality that facilitates the accuracy of classification results. All variant classifications are re-assessed at defined intervals for relevant updates that may influence the interpretation of the final report. Final reports are reviewed and approved according to clinical indications from the research director.

References:

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